<Molecular Biology and Information> Biopolymer Structure

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- Biopolymer Structure -

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Scope of Research

Our research aims are to elucidate structure-function relationships of biological macromolecules, mainly proteins, by using X-ray diffraction method and other physicochemical methods. The following attempts have been mainly made in our laboratory for that purpose. (1) X-ray diffraction studies on protein structures in crystal and in solution are carried out by crystallographic and/or small-angle X-ray scattering techniques to elucidate structure-function relationships of proteins. (2) Molecular mechanism for myosin assembly is studied by proteolytic method, electron microscopy, and computer analysis of the amino acid sequence.

Research Activities (Year 2002)

Presentations

Structure-based analysis of functional sites of thermostable aspartase, Fujii T, Sakai H, Kawata Y (Tottori), Hata Y, XIX Congress and General Assembly of the International Union of Crystallography, 7 - 10 August.

Crystal structures of pokeweed lectins; Hayashida M, Fujii T, Ishiguro M (Kyusyu), Hata Y, Annual Meeting, Jpn. Soc. Biosci. Biotech. AgroChem., 26 March; Annual Meeting, Prot. Sci. Soc. Jpn., 14 June, Kyushu Symp. on Struct. Funct. Prot. Enzy., 18 July, XIX Congress and General Assembly of the International Union of Crystallography, 12 - 15 August, Annual Meeting, Jpn. Biochem. Soc., 16 October, Annual Meeting, Crystallogrr. Soc. Jpn., 11 December.

Detection of Change in Protein Quaternary Structure by Scattering Method: Observation of Degradation and Reconstitution of Chaperonin Proteins GroEL & GroES, Hiragi Y, Ichimura K (Dokkyo), Seki Y (Nagoya), Higurashi T (Osaka), Kawata Y (Tottori), Soda K (Nagaoka), XII International Conference on Small-Angle Scattering, 25 - 29 August.

Structure of ADP dependent thermophilic bacteria *Pyrococcus horikoshii*, Kujime A (Tokushima), Hiragi Y, Tsuge H (Tokushima), Ichimura K (Dokkyo), Goda, S (Tokushima), Sakuraba H (Tokushima), Ohshima, T (Tokushima), Annual Meeting, Jpn Biochem. Soc., 14 - 17 October.

Role of electrostatic interactions in myosin filament formation, Akutagawa T, Hata Y, Ooi T and Katayama E (Tokyo), Annual Meeting, Bophys. Soc. Jpn., 4 November.

Grants

Fujii T, Elucidation of reaction mechanism of high-active-thermostable aspartase by crystallographic analyses of complexes, Grant-in-Aid for Encouragement of Young Scientists, 1 April 2000 - 31 March 2003.

Detection of Change in Protein Quaternary Structure by Scattering Method: Observation of Degradation and Reconstitution of Chaperonin Proteins GroEL & GroES

Although light scattering, CD, fluorescence and sedimentation have so far utilized monitoring the structural change of oligomeric proteins, no direct detection of the quaternary structure was possible by these methods. The Kratky plot of small-angle scattering intensities can assign the structure. State of the denatured entity is also estimable from the pair wise plots of forward scattering intensities and z-average radii of gyration.

1) On GroEL denaturation, peaks on the Kratky plots disappeared at the GdnHCl concentration of 0.8M. From the calculation based on the atomic co-ordinates, it was proved that the peaks originated in the quaternary structure of the tetradecameric oligomer. Pair wise plots of change in forward scattering intensities and z-average radii of gyration obtained from SAXS measurements indicate GroEL 14-mer directly denatured to unfolded coil without passing through the globular monomer in the present case.

2) Denaturation of GroES by GdnHCl showed that the quaternary structure was lost at 1.2M GdnHCl. Denatured coil structured GroES was completely reconstructed to heptameric native oligomer by diluting the GdnHCl judged by the coincidence of radius of gyration and profiles of Kratky plot and distance distribution function between original native structure and that of reconstituted one.

3) In the case of heat denaturation, GroES was denatured at 62 °C and also reconstructed, in the sense of structure, by lowering the temperature.

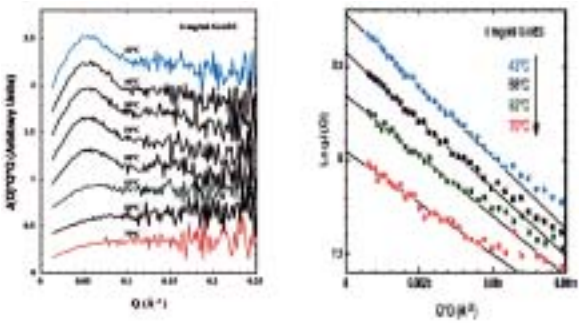

Figure 1. Heat dissociation/unfolding and refolding/reassociation of GroES. The solution condition is 0.5M GdnHCl with 20mM phosphase buffer (pH 7.0).

Role of electrostatic interactions in myosin filament formation

In myosin assembly into thick filaments, the myosin molecules are packed with axial staggers of 14.3 nm and 43 nm. In order to define how these staggers are caused between the rod segments of the adjacent molecules, energy calculation of electrostatic interaction between parallel two rod fragments, when the one fragment shifts along the other, was performed using the rod structure (α-helical coiled coil) and the amino acid sequence of the rod fragment. This amino acid sequence is highly repetitive by alternate clusters of positive and negative charge. Energy of the electrostatic interaction is based on the Deby-Huckkel electrostatic potentials, and sum of these potentials is represented as difference of free energy between dimer formation and monomer state of the two fragments, according to procedure which was developed by Ooi. Obtained energy profile shows that a peak as the lowest free energy appears at shift of 14.3 nm. And also, a lower peak appears at shift of 43 nm. From these lower free energies, the most stable dimer is formed through these shifts, then the most stable thick filament would be formed through staggers of 14.3 nm and 43 nm. Consequently, it is strongly suggested that electrostatic interaction plays a crucial role for these staggers. This work is final achievement of Professor Dr. Tatsuo Ooi, Professor Emeritus of Kyoto University, who passed away on 25th September 2002.

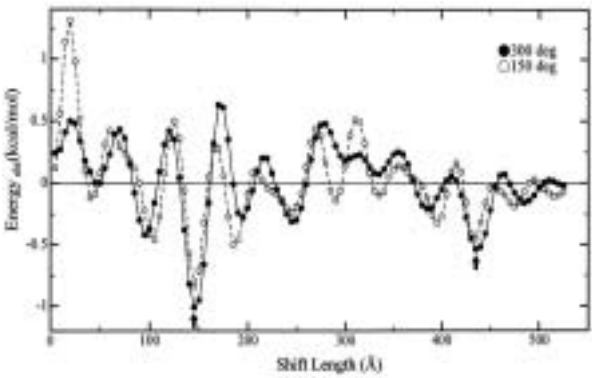

Figure 2. Electrostatic interaction between two parallel rod fragments when the one fragment shifts along the other. This fragment has ability to form paracrystal with a structural repeat of 43 nm. Interaction energy is caused by surface charge clusters of the fragments. Lower values of energy correspond to stronger electrostatic attractions.